which the sample is not passed through a pump, syringe, or tubing prior to or during delivery to the sorting device. In a specific example, the microfluidic device includes an on-chip sample reservoir located upstream from the processing stations such as MFG sorting stations, labeling stations, detection stations, etc. In some cases, sample is preloaded in the reservoir and then consumed during processing in the device. For example, sample flow in the device may be actuated by applying pressure to the reservoir to drive sample out of the reservoir and into the remainder of the device. Using this approach, the sample need never contact a pump or tubing associated with the microfluidic device. Of course, the sample must be delivered to the reservoir prior to the sorting/separation process. Many low loss processes such as pipetting are available for this purpose and known to those of skill in the art.

[0139] Generally, the reservoir should be sized to hold sufficient sample to allow the sorting and any other processes to run to completion on the device. In specific examples, integrated devices such as those exemplified in FIGS. **7**A-**7**D may employ an upstream sample reservoir as described here.

[0140] The nucleic acid amplification technique described here is a polymerase chain reaction (PCR). However, in certain embodiments, non-PCR amplification techniques may be employed such as various isothermal nucleic acid amplification techniques; e.g., real-time strand displacement amplification (SDA), rolling-circle amplification (RCA) and multiple-displacement amplification (MDA).

[0141] Regarding PCR amplification modules, it will be necessary to provide to such modules at least the building blocks for amplifying nucleic acids (e.g., ample concentrations of four nucleotides), primers, polymerase (e.g., Taq), and appropriate temperature control programs). The polymerase and nucleotide building blocks may be provided in a buffer solution provided via an external port to the amplification module or from an upstream source. In certain embodiments, the buffer stream provided to the sorting module contains some of all the raw materials for nucleic acid amplification. For PCR in particular, precise temperature control of the reacting mixture is extremely important in order to achieve high reaction efficiency. One method of on-chip thermal control is Joule heating in which electrodes are used to heat the fluid inside the module at defined locations. The fluid conductivity may be used as a temperature feedback for power control.

[0142] In order to effectively amplify nucleic acids from some pathogens or other target components, the microfluidics system may include a cell lysing or viral protein coat-disrupting module to free nucleic acids prior to providing the sample to an amplification module. Cell lysing modules may rely on chemical, thermal, and/or mechanical means to effect cell lysis. Because the cell membrane consists of a lipid double-layer, lysis buffers containing surfactants can solubilize the lipid membranes. Typically, the lysis buffer will be introduced directly to a lysis chamber via an external port so that the cells are not prematurely lysed during sorting or other upstream process. However, in some cases, the target to be sorted from a sample using labeled magnetic particles is only accessible after lysis. In such cases, it may be necessary to include a lysis module upstream from a sorting module. In such cases, the aim of lysis is to release the intracellular organelles and proteins for magnetophoretic separation processes. In cases where organelle integrity is necessary, chemical lysis methods may be inappropriate. Mechanical breakdown of the cell membrane by shear and wear is appropriate in certain applications. Lysis modules relying mechanical techniques may employ various geometric features to effect piercing, shearing, abrading, etc. of cells entering the module. Other types of mechanical breakage such as acoustic techniques may also yield appropriate lysate. Further, thermal energy can also be used to lyse cells such as bacteria, yeasts, and spores. Heating disrupts the cell membrane and the intracellular materials are released. In order to enable subcellular fractionation in microfluidic systems a lysis module may also employ an electrokinetic technique or electroporation. Electroporation creates transient or permanent holes in the cell membranes by application of an external electric field that induces changes in the plasma membrane and disrupts the transmembrane potential. In microfluidic electroporation devices, the membrane may be permanently disrupted, and holes on the cell membranes sustained to release desired intracellular materials released.

[0143] When the target is a virus or a component of a virus, it may be necessary to disrupt the viral protein coat at some stage in the microfluidic system. This may be done via thermal or chemical means as described for the lysis chamber, bearing in mind that different conditions may be required to remove or compromise a protein coat. In one example, the genetic contents of a virus are extracted by contact with an SDS (sodium dodecyl sulfate) solution. In certain embodiments, viruses coupled to magnetic particles are temporarily immobilized during sorting and/or extraction/separation of viral genetic materials.

[0144] As many viruses are retroviruses (their genetic material is RNA, rather than DNA), it may be necessary to perform reverse transcription in a microfluidic module prior to detection and/or amplification. Reverse transcription may be performed by implemented in a microfluidic module by delivering deoxyribonucleotides, primer, and a reverse transcriptase in a buffer at an appropriate temperature to cause the reverse transcription reaction to proceed. In some cases, reverse transcription and amplification may take place in a single module or station that employs all the necessary components for reverse transcription and amplification. In some embodiments, both processes are implemented by controlling the sequence of delivery of the appropriate nucleosides and enzymes to the station or module.

[0145] Many suitable detection techniques are available to detect target or other species in microfluidic modules employed in embodiments of the invention. These techniques may involve signals that are primarily optical, electrical, magnetic, mechanical, etc. A microfluidic detection module may employ continuous flow of the target or it may employ immobilized target as in the case of a nucleic acid microarray. In certain embodiments, fluorescent detection is employed. This of course requires that a fluorophore be coupled to the target species in or upstream from the detection module (unless the fluorophore is present in the native target as would be the cases with an expressed fluorophore such as a green fluorescent protein). In some embodiments, a detection module includes an inlet for receiving a fluorescently labeled antibody or other component specific for the target or a target associated feature such as a binding moiety on a magnetic particle or a particular protein on cell that carries the target. Presence of the fluorophore in the detection module is detected by exciting the molecule or moiety with light of an appropriate excitation frequency and detecting emitted light intensity at a signature emission frequency.